

A methodCLAIMS

1. ~~Method~~ for screening substances capable of having therapeutic action in the treatment of transmissible subacute spongiform encephalopathies (TSSEs), characterized in that it comprises the following steps:
- 5 a) inoculation at time t_A , into at least one laboratory animal selected from the group consisting of rodents, by any appropriate route, of a nonconventional transmissible agent (NCTA);
- 10 b) administration to the said laboratory animal, by any appropriate route, of either a substance to be screened (test animal), or of a placebo (negative control animal), within a period between t_A - 15 days and t_C , corresponding to the time when the PrPres level in the spleen of the said laboratory animal is at maximum or within a period between t_B , corresponding to the time of the first detection of PrPres in the spleen of the said laboratory animal and t_C ; t_B being between
- 15 t_A and $t_A + 15$ and t_C being between $t_A + 25$ and $t_A + 30$;
- 20 c) sacrificing of the animals within a time interval between t_B and t_C , preferably at t_C , and collecting of the spleen, t_A , t_B and t_C being expressed in days;
- 25 d) isolation of the PrPres from each spleen collected, according to a suitable method of isolation comprising the homogenization of the spleen, followed by a specific extraction of the PrPres comprising a single separation step, from the homogenate obtained,
- 30 and optionally the purification of the PrPres;
- e) semiquantification of the PrPres obtained in step (d) by detection of the said PrPres by any appropriate method, producing a specific signal, followed by a comparison of the signal obtained with a calibration series of dilutions of a positive control consisting of a brain homogenate from an animal at the terminal stage of the disease; and
- 35 f) selection of the screened substances as a candidate for the treatment of transmissible subacute

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spongiform encephalopathies, if the PrPres level obtained in the spleen of the test animal, in step e), is reduced by at least a factor of 2 compared with the level obtained under the same conditions with the negative control animal.

2. ~~A method~~ Method of screening according to Claim 1, characterized in that in step a) the said NCTA is preferably administered in a buffer suited to the route of administration selected in the form either of a crude tissue, preferably brain, homogenate, or of a PrPres pellet, obtained by appropriate centrifugation, from a crude tissue, preferably brain, homogenate.

3. Method of screening according to Claim 1 or Claim 2, characterized in that in step a) the said NCTA is administered by the intraperitoneal route, at a dose corresponding to an inoculum of NCTA, between 0.001% and 10% (weight/volume) (LD_{50} between 10^3 and 10^7).

4. Method of screening according to any one of Claims 1 to 3, characterized in that in step d) the said method of isolation is selected such that the ratio: maximum level detectable in the spleen/cut off is greater than 2 or such that a 1/2 dilution of the final sample obtained still provides a detection signal.

5. Method of screening according to any one of Claims 1 to 4, characterized in that in step d) the said method of isolation of the PrPres comprises a separation in a single step.

6. Method of screening according to any one of Claims 1 to 4, characterized in that in step e) the PrPres is detected by immunoassay.

7. Method of isolating PrPres, from an organ or a tissue, in particular the spleen or the brain, which is capable of being used in a method according to any one of Claims 1 to 6, characterized in that it comprises essentially the following steps:

(i) homogenization of organ or tissue, collected after sacrificing the animal, by mechanical grinding in a homogenization buffer, followed by

calibration of the homogenate, for the production of a homogenate comprising, in weight/volume, from 5 to 50% of the said organ or tissue;

(ii) specific extraction of PrPres comprising a single separation step, by treating the homogenate obtained in step (i) by incubating the suspension obtained with a protease and an anionic detergent capable of promoting the aggregation of the PrPres in a suitable buffer and separation of the PrPres, by a single ultracentrifugation at 480,000-1,200,000 g.h, preferably for 2-4 hours, for example at 240,000-300,000 g for 2 to 4 h, preferably at 20-22°C, of the said suspension, deposited on a buffer cushion having a density of between 1.02 and 1.08, at 20°C and recovering the centrifugation pellet comprising the said PrPres; and, if necessary,

(iii) purification of the PrPres by suspending the centrifugation pellet obtained in (ii) in a Laemmli buffer comprising 1-5% SDS, incubating in this buffer at 100°C for 2-10 minutes and centrifuging at 12,000-15,000 g for 10-15 minutes at 16-22°C.

8. Method of isolating PrPres, from an organ or a tissue, in particular the spleen or the brain, which is capable of being used in a method according to any one of Claims 1 to 6, characterized in that it comprises essentially the following steps:

(i) homogenization of organ or tissue, collected after sacrificing the animal, by mechanical grinding in a homogenization buffer, followed by the addition, to the homogenate obtained, of a salt having a high ionic strength and capable of promoting the aggregation of the PrPres in a 1:1 (v/v) ratio, followed by calibration of the homogenate, for the production of a homogenate comprising, in weight/volume, from 5 to 50% of the said organ or tissue;

(ii) specific extraction of PrPres by treating the homogenate obtained in step (i) by incubating the suspension obtained with solution comprising a protease

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and an anionic detergent capable of promoting the aggregation of the PrPres, and a single separation of the PrPres, by centrifugation at 25,000-60,000 g.h, for example at 25,000-30,000 g for 1 to 2 h, preferably at 16-22°C, of the suspension obtained, deposited on a buffer cushion having a density of between 1.02 and 1.08, at 20°C, and recovering the centrifugation pellet comprising the said PrPres; and, if necessary,

(iii) purification of the PrPres by suspending the centrifugation pellet obtained in (ii) in a Laemmli buffer comprising 1-5% SDS, incubating in this buffer at 100°C for 2-10 minutes and centrifuging at 12,000-15,000 g for 10-15 minutes at 16-22°C.

9. Method according to Claim 7 or 8, characterized in that the homogenization buffer in step (i) is in particular a neutral buffer such as water or an isotonic buffer such as 5% glucose.

10. Method according to Claim 7 or Claim 8, characterized in that in step (ii), prior to the centrifugation, at least one protease inhibitor is added.

11. Method according to Claim 7 or Claim 8, characterized in that in step (ii) the centrifugation is preferably carried out after depositing the suspension containing the PrPres on a 6-20% sucrose cushion.

12. Method according to Claim 8, characterized in that during the extraction step (ii) the solution used for the extraction comprises an anionic detergent capable of promoting the aggregation of the PrPres and a zwitterionic detergent, such as a sulphobetaine, preferably the sulphobetaine SB 3-14 at 1-2%, in a 1:1 (v/v) ratio.

13. Method according to Claim 8, characterized in that in the extraction step (ii) the centrifugation is preferably carried out after depositing the suspension containing the PrPres on a cushion comprising, in a mixture, 6-20% sucrose and a sulphobetaine.

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14. Application of ~~a~~ method according to any one of Claims 7 to 13 to the detection of PrPres in an organ or a tissue.

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